COMPARISON BETWEEN NUMBER OF NERVE FIBERS IN NORMAL BREAST TISSUE, BENIGN LESIONS AND MALIGNANT BREAST TUMORS

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Abstract- Breast cancer is common and is considered second cause of cancer related mortality in females. Regarding importance of breast cancer, more investigation in this field is recommended. For many years investigators believed that neoplasms were not innervated but new findings have proved otherwise. This descriptive study was carried out to compare number of nerve fibers in benign, malignant and normal breast tissue. Of each group several slides were reviewed and 3608.50 mm^2 of malignant tumors (ductal carcinoma), 3641 mm^2 of benign tumors (fibroadenoma) and 2331.25 mm^2 of normal breast tissue (mammoplasty) were assessed. Numbers of nerve fibers were compared and a significant increase in nerve fibers was found in malignant tumors compared with benign tumors and normal breast tissue. Accuracy of hematoxylin and eosin method were examined by immunohistochemistry staining (neurofilament) method and affirmed. These results reveal that malignant tumors of breast have more nerve fibers than normal breast tissue or benign tumors.

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INTRODUCTION

Breast cancer is common and is considered second cause of cancer related mortality in females. Regarding importance of breast cancer, more investigation in this field is recommended and innervation of tumors is a new source of investigation in this field.

For many years investigators believed that neoplasms were not innervated, but today with the aid of newer methods and more investigations this idea is under question. Many researches on different tumors, including pancreatic cancers (1), parathyroid gland adenoma (2), hepatocellular carcinoma and cholangiocarcinoma (3) have paid attention to innervation of the tumors.

There are some studies that show evidences of presence of nerve fibers in breast tumors (4), pigmented and non pigmented adenoma of the ciliary body (5), and prostate (6).

In accordance with these studies, an assay on prostate by one of the authors showed an increase of nerve fibers in malignant prostatic lesions compared to benign lesions. A similar study on prostate by Zhou *et al.* showed decrease of nerve fibers in malignant lesions compared to benign lesions (6), but they had used benign tissue around the malignant lesion for control.

We decided to compare the number of nerve fibers in normal breast tissue and malignant and benign breast tumors and confirm the results by immunohistochemistry staining.

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MATERIALS AND METHODS

Three groups were selected randomly: 50 patients with malignant breast lesions, 50 patients with benign breast lesions and 6 patients who underwent mammoplasty surgery as normal group. We obtained informed consent from all subjects.

Methods of biopsy in malignant group were 23 excisional biopsy, 25 mastectomies, one lumpectomy and one quadrantectomy. In addition to 25 mastectomy samples we had access to axillary lymph nodes of three other cases; so we assessed axillary lymph nodes of 28 cases.

Slides with necrosis were excluded and finally 50 slides of malignant group, 50 slides of benign group and 42 slides of normal group were chosen. They were stained by hematoxylin and eosin (H & E) method and to simplify measuring a rectangle figure was drawn on each slide by a fine pen, so that tissue occupied its entire surface. Surfaces were measured and marked areas were assessed carefully by x40 objective lens of microscope and nerve fibers were counted.

To assess precision of our H & E investigation, five slides of each group were chosen randomly and neurofilament (NF) immunohistochemistry staining were done. All the H & E slide area were assessed first and nerve fibers number were written and then NF slides were seen.

Results were assessed by SPSS software. $P < 0.05$ was considered significant.

RESULTS

We obtained samples from 50 patients with malignant breast lesions, 50 patients with benign breast lesions and 6 patients who underwent mammoplasty surgery as normal group. All malignant lesions were invasive ductal carcinoma and all benign lesions were fibroadenoma.

Fifty benign, 50 malignant and 42 normal slides were assessed. Age range was 12-50 years with a mean of 27.4 years in benign group, 27-75 years with a mean of 46.9 years in malignant group and 21-41 years with a mean 31 years in normal group.

Of total of 100 cases of benign and malignant lesions, site of lesion was unknown in 4 cases. In remaining 96 cases, there was equal number of right and left side lesions. In normal group we obtained slides from both right and left sides.

Positions of malignant tumors were not clear in 26 cases. Of remaining lesions, 2 were located in subareolar region, 11 in upper outer quadrant (UOQ), 5 in upper inner quadrant (UIQ), 4 in lower outer quadrant (LOQ) and 2 in lower inner quadrant (LIQ). Position of 23 benign lesions were not clear; of 28 remaining lesions 5 were located in subareolar region, 16 in UOQ, 4 in UIQ, 1 in LOQ and 1 in LIQ.

Nuclear and histologic grades were determined based on Nottingham Modification of Bloom and Richardson (7). Nuclear grading showed that 13 samples were in grade I; 27 in grade II and 10 in grade III. Histologic grading revealed that 5 samples were in grade I, 23 in grade II and 22 in grade III.

Perineuronal and vascular invasion were assessed too. Because of unavailability of all the tumoral masses, we could not have a definite idea about invasion. There were perineuronal invasion in 13 cases and vascular invasion in 29 cases.

We assessed axillary lymph nodes of 28 cases. Of these, five cases were free of lymph node tumoral involvement. Of 23 cases with lymph node involvement, histologic grade were as below: 14 cases grade III and 9 cases with grade II.

Total assessed slide area was 3608.50 mm^2 in malignant group, 3641 mm^2 in benign group and 2331.25 mm^2 in normal group (Table 1).

There were 14 nerve fibers in malignant group (maximum 3 and minimum zero nerve fiber in each slide) (Figures 1 and 2). One nerve fiber was found in benign group (Figures 3 and 4) and one in normal group. The proportions of number of nerves to area were calculated (Table 1).

Table 1. Number of Nerve fibers, total area and their proportion due to diagnosis*

Diagnosis	Number of nerves	Total area (mm^2)	Number of nerves/area
Malignant	14	3608.50	0.0039
Benign	1	3641	0.0003
Mammoplasty	1	2331.25	0.0004

* There was a significant difference between nerve fibers in malignant and benign tumors and between normal and malignant group.

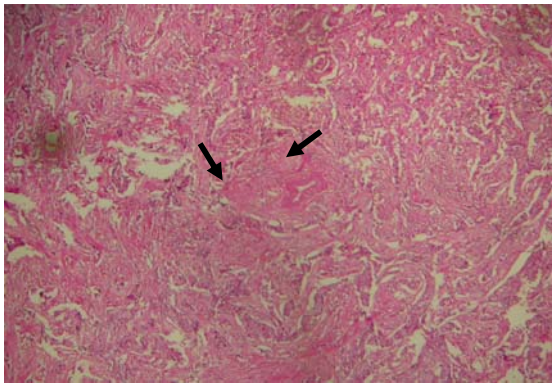

Fig. 1. Cross section of two nerve fibers (arrows) found in invasive ductal carcinoma of the breast. H & E (X100).

Results of statistical analysis, using analysis of variance (ANOVA) and Chi square, showed 1) significant difference between nerve fibers in malignant and benign tumors (P = 0.021), 2) significant difference between normal and malignant group (P = 0.027), 3) no significant difference between benign and normal group ($P > 0.05$), 4) no significant difference between nuclear and histologic grade and nerve fibers number, and 5) a positive correlation between histologic grade and lymph node involvement (P = 0.005).

Numbers of nerve fibers in 5 randomly chosen slides of malignant group were three nerves in H & E slides and three in NF (Fig. 5). Two of specimens were the same but one didn't have as much tissue as the other slide. So we compared two of them and there was 100% matching between two staining methods. In 10 slides of two other groups we didn't find any nerve fiber in either of staining methods.

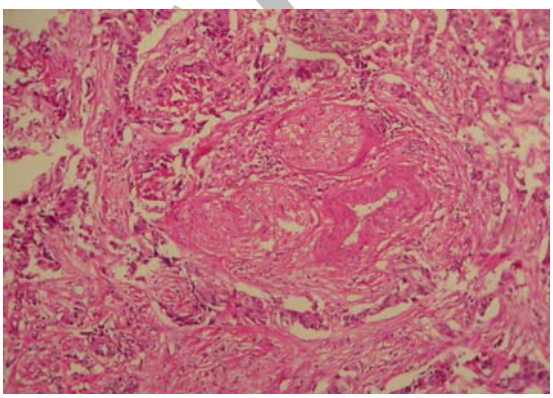

Fig. 2. Nerves showed in Fig1. H & E (X400).

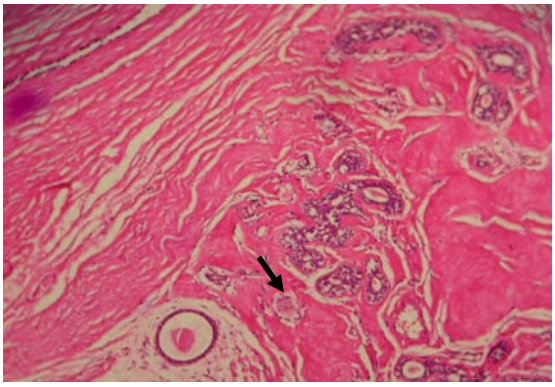

Fig. 3. Cross section of the nerve (arrow) found in fibroadenoma H & E (X100).

DISCUSSION

Breast diseases are very common in females. Although benign lesions are more frequent, malignant tumors are among the most common cancers and are the second common cases of malignancy related mortality in females (8). Incidence of breast cancer in our country is the same as other countries but it seems that age of onset is lower in our country (annual report of Imam Khomeini Medical Center, hospital based cancer registry, 1995). Concerning the importance of breast cancer, more investigation in this field is recommended.

Like angiogenesis of the tumors, their innervation is a new source of investigation and may have clinical uses. There are a few studies on innervation of tumors and only one descriptive study was found on the breast.

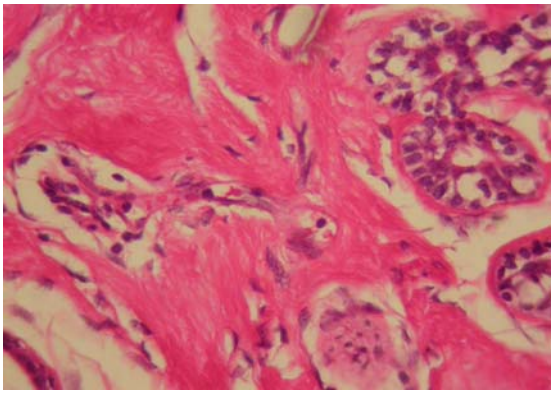

Fig. 4. Nerve showed in Fig 3. H & E (X400).

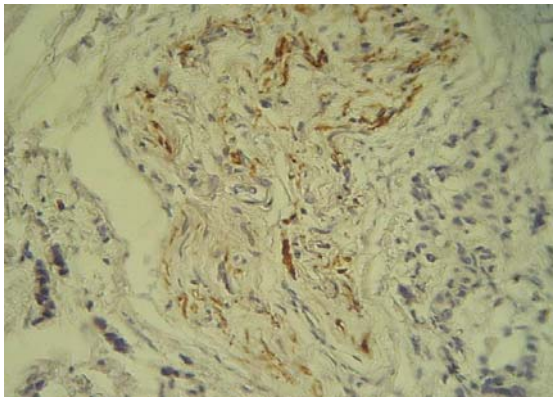

Fig. 5. Nerve fiber found in invasive ductal carcinoma. Neurofilament IHC stain (X400).

This study was a pilot study to compare nerve fibers in malignant, benign, and normal breast tissue. This investigation showed that breast tumors are innervated and confirmed the new findings concerning innervation of tumors.

According to our results, the countable nerve fibers in malignant slides were more frequent than benign and normal slides and the precision of counting was proved by IHC staining. It can be proposed as a hypothesis that something like a secretory mediator increases number or diameter of nerve fibers in malignant tumors. In another study done in Iran five years ago, the same results were seen in the prostate samples. It's premature to get a definite conclusion and more studies on more samples, with specific staining must be done.

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